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Mini Review

Application of High-Throughput Sequencing Technology for the Discovery of Insect Viruses

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Abstract

Many virus classes infect various insect species and cause sub-lethal, asymptomatic and latent infections. A number of conventional methods for viral isolation have already been explored but they usually lack sensitivity. In the last decade, the next generation sequencing (NGS) with high-throughput sequencing (HTS) technology became available for new virus discovery and identification of known viruses, with a relatively low cost. HTS technology provides an unmatched opportunity to study insect response to viruses through gene expression analysis. This review briefly discusses several available HTS methods. The study emphasizes on the contribution of high-throughput technology for the discovery of new insect viruses and the auxiliary opportunities to design novel genetic approaches for management of insect infection by viruses.

Keywords: *Insect; Virus; High-Throughput Sequencing*

Abbreviations

NGS: Next Generation Sequencing; HTS: High-Throughput Sequencing

Introduction

Viruses have been found associated with different organisms including human, animals, plants, fungi and bacteria [1]. Today circa 4,958 species of viruses have been defined (International Committee on Taxonomy of viruses 2018, https://talk.ictvonline.org/). Viruses that infect insects are of particular interest as their identification could help protection of beneficial insects from virus infection e.g. the honey-bee *Apis mellifera* L. [2] and for management of pest insects including invasive species e.g. lepidopteran pests [3]. Generally, insects display an abnormal phenotype due to viral infections. Viruses such as baculoviruses (family: *Baculoviridae*) a DNA viruses that infect insect and can cause clear symptoms and finally death of the host while many other viruses can be asymptomatic i.e., colonies of honey bee (*Apis mellifera* L.) are subjected to many persistent viral infections that do not exhibit clinical signs [4]. Such viruses cannot be detected by use of traditional ways for virus discovery as they are present in low titer and traditional methods are time consuming and labor intensive [5]. Recently, with the development of high throughput sequencing it became possible to detect asymptomatic viruses. Theses sequencing methods include an extensive range of application based on high-throughput genome sequencing, transcriptome profiling and small RNA analysis [6].

HTS has a significant role in the discovery of micro-organisms and viruses [7]. Recently, a report has been published by our group on watermelon virome using next generation sequencing technology and distinct viral species have been identified [8]. This scientific knowledge offers a rapid, low budget cost and accurate sequencing for identification of new viruses either from whole insects or specific tissue parts [9,10]. This technology has also advanced the means for the identification of viruses present in low titers, which do not exhibit any symptoms on host [11].

High-throughput sequencing for discovery of insect viruses

Earlier sequencing method was based on Sanger sequencing technique [12] and was referred to as first-generation technology while the contemporary methods are referred to as next-generation sequencing [13]. Number of high-throughput sequencing techniques have been developed so far and most common among them are provided in the table below [14-16].

Company/Platform	Roche /454	Illumina/Hiseq 2000/2500/3000/4000	Life Technologies/ Solid5500	Illumina/Miseq
Clonal amplification	Fragment/emulsion PCR	Bridge amplification	Fragment / emulsion PCR	Fragment/emulsion PCR
Sequencing Principal	Pyrosequencing	Sequencing by synthesis	Sequencing by ligation	Sequencing by synthesis
Read length(bp)	700 - 1000	100	75	2 X 150
Gb/run	0.7	600	300	15
Advantages/ Disadvantages	Low throughput, short run times, high error rate in homo-polymer repeats, insert size up to 1600 bp, high capital cost	Very high throughput, long and short depending on the platform, error rate increase at 3' end, insert size 600 bp, low capital cost	high throughput, long run time, two base encoding provides inherent error correction, insert size 300 bp, high capital cost	High throughput, short run time, low error rate, insert size 300 bp, low capital cost
Application	Bacterial and insect genome *de novo* assemblies, virus discovery in metagenomics	Variant discovery by whole- genome resequencing, virus discovery and gene discovery in metagenomics	Variant discovery by whole-genome resequencing, gene discovery in metagenomics	Perform targeted gene, small genome, and amplicon sequencing, as well as 16S metagenomics and targeted gene expression analysis

Various steps in HTS for viral discovery

High throughput sequencing methodology deals with extraction of the desired nucleic material either total DNA or RNA or enrichment with si/miRNA or mRNA from infected insects [17]. Second step includes the library preparation which further varies according to the platform used for sequencing (Table). Different type of libraries used are provided in figure 1A. Library preparation include fragmentation of appropriate nucleic material, selection of fragment sizes, addition of adapters, PCR methods depending on library and finally amplification of sequences. Various steps in bioinformatics analysis are given in figure 1B. Number of bioinformatics tools and program are available to process raw data into scientific information for discovery of new insect viruses.

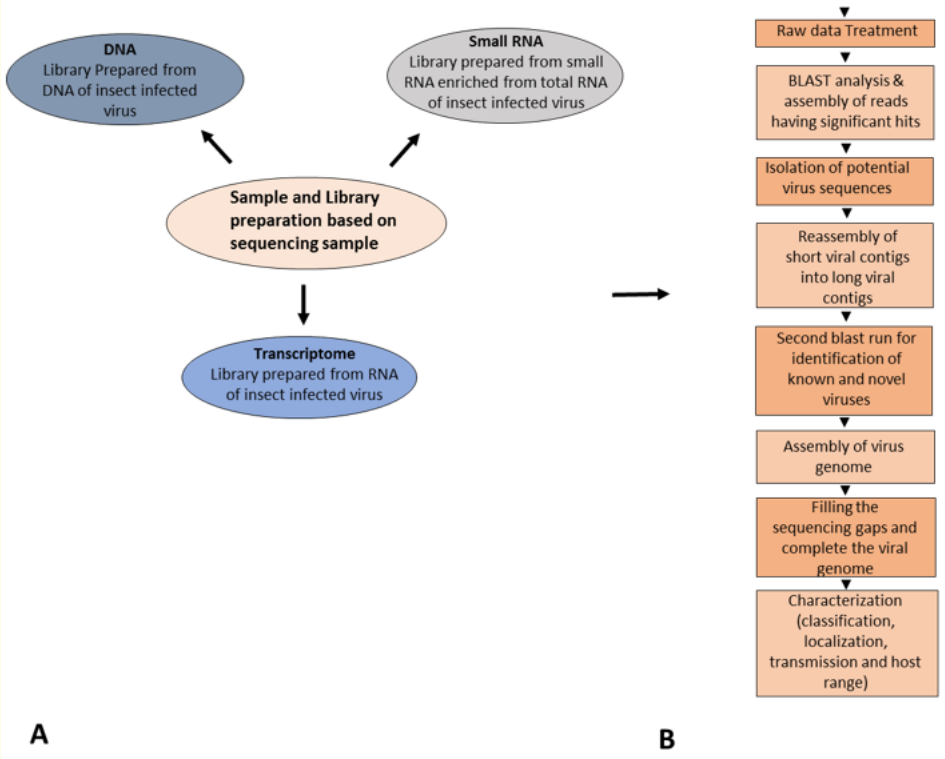

Figure 1: *A. Type of Libraries used in NGS analysis B. Processing steps in bioinformatics analysis.*

Third generation sequencing

Although second generation sequencing has revolutionized the discovery of viruses, this technique has some limitations and one major limitation is that it is purely homology based because novel viruses cannot be identified in the absence of reference genome. Moreover, the second generation technology is time consuming and this platform is costly [18].

Third generation sequencing (TGS) has also been evolved that includes, 1) Single Molecule Real-Time Technology SMART (Pacific Biosciences), this technology used sequencing by synthesis followed by optical monitoring fluorescently tagged nucleotides as they are incorporated into individual template molecule. These platforms generally produces ~100,000 bp with highest throughput of ~8 GB/day [19], 2) Nanopore sequencing technology (Oxford Nanopore Technology), Nanopore devices are electrical based as they sequence DNA by electronically measuring very small disruptions to electric current as DNA pass through Nanopore [20]. TGS is different from its predecessors in two ways; first, it is based on direct detection of nucleotide and there is no need of template amplification prior to sequencing, which reduces template preparation time and coasts. Second, sequencing signals are recorded in real-time during enzymatic reactions. TGS however, is still in a developing stage.

Conclusions

Availability of HTS technology allowed the discovery of new insect viruses and the study of virus-insect interactions as it has also provided a way to explore host response to virus infection. Information on transcriptome data of both infected and non-infected insects can be used to elucidate the host response to gene transcription of viruses. This platform also offers means to analyze susceptibility of

viral RNA to degradation by host RNAi response [21]. Small interfering RNA (siRNA) are produced by dicer enzyme activity in the host insect in response to viral infection and promote the degradation of the viral RNA [22]. In such a case small RNA library preparation and analysis can help to expand the knowledge of promising viruses associated with the host insect. Therefore, RNAi mechanism can be exploited to detect new viruses [23]. Additional improvement of the current technology is apparent in the discovery of novel insect virus, which could be upgraded to third generation sequencing technology.

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